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Estrela, A.B., Abraham, W.-R.
Brevundimonas vancanneytii sp. nov., isolated from blood of a patient with endocarditis
**Brevundimonas vancanneytii** sp. nov., isolated from blood of a patient with endocarditis

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A Gram-negative, rod-shaped, non-spore-forming bacterial strain, designated LMG 2337\textsuperscript{T}, was isolated from the blood of a patient with endocarditis and characterized. The strain was affiliated with the alphaproteobacterial genus *Brevundimonas*, with *Brevundimonas diminuta* LMG 2089\textsuperscript{T} (98.3\% 16S rRNA gene sequence similarity) and *Brevundimonas terrae* KSL-145\textsuperscript{T} (97.5\%) as its closest relatives. This affiliation was supported by chemotaxonomic data: the G+C content was 66.3 mol\%, the major polar lipids were phosphatidyl diacylglycerol, sulfoquinovosyl diacylglycerol and phosphatidyl glycospyranosyl diacylglycerol and the major fatty acids were summed feature 7 (one or more of C\textsubscript{18:1}ω7c, C\textsubscript{18:1}ω9t and C\textsubscript{18:1}ω12t) and C\textsubscript{16:0}. Strain LMG 2337\textsuperscript{T} displayed an unusually broad substrate spectrum. The results from DNA–DNA hybridization and physiological and biochemical tests allowed the genotypic and phenotypic differentiation of strain LMG 2337\textsuperscript{T} from all of the type strains of hitherto-described *Brevundimonas* species. The strain therefore represents a novel species, for which the name *Brevundimonas vancanneytii* sp. nov. is proposed, with type strain LMG 2337\textsuperscript{T} (=CCUG 1797\textsuperscript{T} = ATCC 14736\textsuperscript{T}).

The genus *Brevundimonas* was proposed by Segers et al. (1994) to harbour strains previously assigned to *Pseudomonas diminuta* and *Pseudomonas vesicularis*. At that time, *Brevundimonas* included only species that form short rods that are motile by means of one polar flagellum with a short wavelength. This changed when caulobacteria from a broad range of freshwater, brackish water, marine and soil habitats were studied using a polyphasic approach (Anast & Smit, 1988; MacRae & Smit, 1991; Segers et al., 1994). The descriptions of the genera *Caulobacter* and *Brevundimonas* were emended and a number of *Caulobacter* species were transferred to the genus *Brevundimonas* (Abraham et al., 1999). Today, *Brevundimonas* species are differentiated from *Caulobacter* mainly by the lack of loop 46 in the V8 region of the 16S rRNA gene (Abraham et al., 2008), the presence of C\textsubscript{12:0} 3-OH, the glycoprophospholipid composition and higher salt tolerances. We report here on a novel species within the genus *Brevundimonas* that was isolated from blood of a patient with endocarditis and preliminarily identified as belonging to *Brevundimonas diminuta* (Segers et al., 1994).

The reference strains for this study were obtained from the ATCC, DSMZ and LMG culture collections. The strains were grown in the freshwater *Caulobacter* medium PYEM [per litre MQ-water (Biocel A 10; Millipore): 2 g peptone, 2 g yeast extract, 0.5 g NH\textsubscript{4}Cl]. After autoclaving and cooling, 5 ml filter-sterilized 0.2 mg riboflavin ml\textsuperscript{-1}, 2 ml 50\% glucose, 1 ml 20\% MgSO\textsubscript{4} and 1 ml 10\% CaCl\textsubscript{2} (all sterilized) were added. The strains were grown in 2 l flasks at 30 °C with shaking at 100 r.p.m. and biomass was harvested in the late exponential phase after 72 h.

For the determination of DNA base composition, genomic DNA was isolated from two loopfuls of bacterial cells using the DNeasy Blood and Tissue kit for purification of total DNA (Qiagen) with the addition of RNase A (Sigma), according to the manufacturers’ instructions. DNA was enzymically digested as described by Gehrke et al. (1984) and the mean G+C content was determined by HPLC (Tamaoka & Komagata, 1984). Calculations were performed according to Mesbah et al. (1989), with non-methylated lambda phage DNA (Sigma) as the standard. The G+C content of strain LMG 2337\textsuperscript{T} was 66.3 ± 0.1 mol\%, which is in the range of values already reported for species of the genus *Brevundimonas* (Segers et al., 1994; Vancanneyt et al., 2005; Yoon et al., 2006).

The phylogenetic position of strain LMG 2337\textsuperscript{T} was determined by analysis of the 16S rRNA gene sequence (Abraham et al., 1999) using CLUSTAL W (Thompson et al., 1997). An alignment of sequences from the EMBL database (Kanz et al., 2005) was used to construct a maximum-parsimony tree (Fig. 1) with MEGA version 3.1 (Kumar et al., 2004) and *Hirschia baltica* DSM 5838\textsuperscript{T} as an outgroup. The 16S rRNA gene sequence from strain LMG
2337T lacked loop 46 in the V8 region, which is characteristic for *Brevundimonas* species, and had the highest similarities with *B. diminuta* LMG 2089T (98.3 %) and *Brevundimonas terrae* KSL-145T (97.5 %).

To determine whether strain LMG 2337T belonged to the species *B. diminuta* or *B. terrae* or whether it represented a novel species, DNA–DNA hybridizations between strain LMG 2337T and *B. diminuta* LMG 2089T and *B. terrae* DSM 17329T were performed. DNA was isolated using a French pressure cell and purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out twice as described by De Ley et al. (1970) using the modification described by Huß et al. (1983) in a Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with an in situ temperature probe. The DNA–DNA relatedness between strain LMG 2337T and *B. diminuta* LMG 2089T and *B. terrae* DSM 17329T was 62 and 16 %, respectively. It has been shown that DNA–DNA relatedness between two strains of 70 % or more qualifies them as members of the same species (Wayne et al., 1987). Hence, the results indicated that strain LMG 2337T indeed belonged to a novel species.

![Maximum-parsimony dendrogram of the phylogenetic relationships between strain LMG 2337T and strains of the genus *Brevundimonas*, based on a distance-matrix analysis of 16S rRNA gene sequences. The sequence of *Hirschia baltica* DSM 5838T (GenBank accession no. AJ421782) was used as an outgroup (not shown).](image)

**Table 1.** Fatty acid contents of whole-cell hydrolysates of strain LMG 2337T and some type strains of the genus *Brevundimonas*

<p>| Strains: | 1, <em>B. vacanneytii</em> sp. nov. LMG 2337T; 2, <em>B. diminuta</em> LMG 2089T; 3, <em>B. bullata</em> DSM 7126T; 4, <em>B. subvibrioides</em> LMG 14903T; 5, <em>B. alba</em> ATCC 15265T; 6, <em>B. variabilis</em> ATCC 15255T; 7, <em>B. bacteroides</em> LMG 15096T; 8, <em>B. aurantiaca</em> ATCC 15266T; 9, <em>B. intermedia</em> ATCC 15262T; 10, <em>B. vesicularis</em> LMG 2350T. Values are percentages of total fatty acids; data were obtained in this study. ECL, Equivalent chain-length; tr, trace (&lt;1.0 %); −, not detected. |</p>
<table>
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<th>Fatty acid</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<td>1.5</td>
<td>1.3</td>
<td>2.8</td>
<td>1.1</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
<td>1.7</td>
<td>1.9</td>
</tr>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>2.5</td>
<td>3</td>
<td>3.4</td>
<td>1.5</td>
<td>2.4</td>
<td></td>
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<td>5.1</td>
<td>1.5</td>
<td>5.4</td>
<td>5.4</td>
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<td>4</td>
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<td>−</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>10.8</td>
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<td>53.5</td>
<td>38.7</td>
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<td>56.7</td>
<td>43.2</td>
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<td>−</td>
<td>−</td>
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<td>−</td>
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<td>−</td>
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<tr>
<td>11-Methyl <em>C</em>18 : 1&lt;sup&gt;ω5t&lt;/sup&gt;</td>
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<td>−</td>
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<td>2.7</td>
<td>−</td>
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<td>3.5</td>
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<td>7.4</td>
<td>6.2</td>
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<td>−</td>
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<td><em>C</em>20 : 1&lt;sup&gt;ω6,9c&lt;/sup&gt;</td>
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<td>−</td>
<td>−</td>
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</table>

*Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 4 consisted of one or more of iso-*C*15 : 0 2-OH, *C*16 : 1<sup>ω7c</sup> and *C*16 : 1<sup>ω7t</sup>. Summed feature 7 consisted of one or more of *C*18 : 1<sup>ω7c</sup>, *C*18 : 1<sup>ω9t</sup> and *C*18 : 1<sup>ω12t</sup>.

†11-Methyl-12-trans-octadecanoic acid has ECL 18.080.
For whole-cell fatty acid analysis, cells were grown at 30 °C for 48 h on PYEM agar plates (1.5 % agar). Cells were saponified (15 %, w/v, NaOH at 100 °C for 30 min), methylated to fatty acid methyl esters (methanolic HCl at 80 °C for 10 min) and extracted (hexane/methyl tert-butyl ether, 1:1, v/v) as described in detail by Osterhout et al. (1991). Fatty acid methyl esters were analysed on a gas chromatograph equipped with a fused-silica capillary column (25 m × 0.2 mm) with cross-linked 5 % phenyl methyl silicone (film thickness 0.33 μm; HP Ultra 2) using the protocol of Osterhout et al. (1991). For all strains, the main fatty acids were summed feature 7 (one or more of C₁₈:1ω7c, C₁₈:1ω9t and C₁₈:1ω12t) and C₁₆:0 and the main hydroxy fatty acid was C₁₂:0 3-OH, all of which are characteristic of the genus _Brevundimonas_ (Table 1).

Polar lipid analysis with fast atom bombardment mass spectrometry was performed in the negative mode on the first instrument in an E₂B₁E₂B₂ configuration (JMS-HX/HX110A; JEOL) using conditions described by Abraham et al. (1997). All strains contained phosphatidylglycerol, 1,2-di-O-acyl-3- O-[β-D-glucopyranosyl-(1→4)-α-D-glucopyranuronosyl] glycerol (DGL), lyso-phosphoglycolipid and 3-O-[6’S -(sn-glycero-3’-phosphoryl)-α-D-glucopyranosyl]-sn-glycerol. The almost exclusive occurrence of C₁₉:1 DGL and the almost exclusive absence of C₁₈:1 DGL in strain LMG 2337⁺ are remarkable. Furthermore, sulfoquinovosyl diacylglycerol was detected in strain LMG 2337⁺ (Table 2).

For phenotypic characterization, strains were grown at 30 °C in 20 ml PYEM amended with 0, 5, 10, 20, 30, 40, 60, 80 or 100 g NaCl l⁻¹. The OD₆₀₀ of each cell suspension was determined at the beginning of the experiment and after 2 days and the difference between the two measurements was used to determine salt tolerance. Strain LMG 2337⁺ grew best with 5–30 g NaCl l⁻¹ and grew slowly with 60 g NaCl l⁻¹ but not with 80 g NaCl l⁻¹.

**Table 2. Phospholipids and sulfolipids in strain LMG 2337⁺ and some type strains of the genus *Brevundimonas***

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<th>Mass (Da)</th>
<th>Polar lipid</th>
<th>COOH</th>
<th>Present in strain:</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
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<td>PG</td>
<td>C₁₈:1</td>
<td>C₁₅:0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>+</td>
<td>-</td>
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<td>C₁₆:1</td>
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<td>-</td>
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Substrate specificity tests were conducted with API 20NE test strips (bioMérieux) and GN2 MicroPlates (Biolog), according to the manufacturers’ instructions, at 30 °C for 24 h. A test was considered positive if the interface between the sample well and the air was visibly turbid due to bacterial growth (Rüger & Krambeck, 1994). The results are given in the species description and in Table 3. It should be noted that strain LMG 2337T showed an exceptionally broad substrate usage, which differed sharply from that of the reference strains. Enzyme activity tests were conducted with API ZYM test strips (bioMérieux), according to the manufacturer’s instructions. The test for leucine arylamidase activity was weakly positive in strain LMG 2337T but positive in B. diminuta LMG 2089T. The characters that discriminate between strain LMG 2337T and its closest phylogenetic neighbours are summarized in Table 3.

### Table 3. Characteristics that are useful for distinguishing strain LMG 2337T from closely related type strains of the genus *Brevundimonas*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<td><strong>Enzyme activity</strong></td>
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<tr>
<td>Leucine arylamidase</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Valine arylamidase</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>++</td>
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<td>Acid phosphatase</td>
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<td>Protease</td>
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<td><strong>Substrate utilization</strong></td>
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<tr>
<td>Arabinose</td>
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<td>D-Galactose</td>
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<td>Maltose</td>
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<td>D-Mannose</td>
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<td>Malate</td>
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<td>Quinic acid</td>
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<td>3-Cyclodextrin</td>
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<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>66.3</td>
<td>61.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.7&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

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<sup>a</sup>Data from: <i>a</i>, Yoon et al. (2006); <i>b</i>, Segers et al. (1994); <i>c</i>, Fritz (2000).
Strain LMG 2337<sup>T</sup> could be differentiated from all hitherto-described *Brevundimonas* species. In particular, strain LMG 2337<sup>T</sup> differed from *B. terrae* DSM 17329<sup>T</sup> by having strong acid phosphatase activity and the ability to use z-keto-fatty acids and many amino acids, from *B. diminuta* LMG 2089<sup>T</sup> by having smaller amounts of C<sub>17:1</sub>ω6c and C<sub>17:1</sub>ω8c, the ability to use p-hydroxyphenylacetic acid, d-gentiobiose, d-alanine and adonitol and from *Brevundimonas bullata* DSM 7126<sup>T</sup> by the ability to use many amino acids. As a consequence, we propose a novel species, *Brevundimonas vancanneytii* sp. nov.

**Description of *Brevundimonas vancanneytii* sp. nov.**

*Brevundimonas vancanneytii* (van.can.ney’ti.i. N.L. masc. gen. n. *vancanneytii* of Vancanneyt, named after Marc Vancanneyt, a microbiologist in Ghent, Belgium, who has contributed to the chemotaxonomy of many genera).

Cells are short rods and motile by one polar flagellum with no prosthecae. Cell division is by fission. Colonies are whitish yellow. Grow on peptone yeast extract medium with 0–60 g NaCl<sup>−1</sup> but not with 80 g NaCl<sup>−1</sup>. Utilizes z-(+)-d-glucose, β-(+)-d-fructose, (+)-d-galactose, (+)-trehalose, (+)-d-mannose, (+)-methylglucoside, sucrose, (+)-raffinose, maltose, lactose, d-psicosé, (+)-gentiobiose, (+)-l-arabinose, z-l-rhamnose, z-l-fucose, (+)-turanose, (+)-d-arabitol, glycerol, myo-inositol, D-mannitol, d-sorbitol, adonitol, d-saccharic acid, malic acid, cis-aconitic acid, citric acid, d-glucuronic acid, d-galacturonic acid, d-gluconic acid, p-hydroxyphenylacetic acid, (−)-quinic acid, 4-aminoacbutyric acid, DL-lactic acid, succinic acid, capric acid, itaconic acid, pyruvic acid methyl ester, succinic acid monomethyl ester, z-, β- and γ-hydroxybutyric acids, propionic acid, acetic acid, bromocrescic acid, z-ketobutyric acid, z-ketoglutaric acid, z-ketovaleric acid, formic acid, malonic acid, sebacic acid, d-galactonic acid lactone, D-glucosaminic acid, urocanic acid, succinic acid, L-pyrogulamic acid, L-aspartic acid, L-glutamic acid, glycolyl L-glutamic acid, glycolyl L-aspartic acid, L-alanyl glycine, hydroxy-L-proline, L-threonine, L-leucine, L-histidine, L-asparagine, L-proline, D- and L-alanine, D- and L-serine, L-ornithine, L-phenylalanine, N-acetyl-D-glucosamine, putrescine, 2-amineoethanol, alanimamide, Tweens 40 and 80, methyl d-glucoside, dextein, glucuronamidase, uridine, 2,3-butanediol, DL-z-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate. Does not utilize glycogen, inosine, thymidine, ascinulin, arginine, urea or tryptophan. Nitrate is not reduced. Produces alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, z-chymotrypsin, naphthol-AS-BI-phosphohydrolase and oxidase, but not catalase. The polar lipids are z-D-glucopyranosyl, z-D-glucuronopyranosyl, β-D-glucopyranosyl-(1→4)-z-D-glucopyranosyluronosyl, sulfoquinovosyl, phosphatidyld- and 6-phosphatidyl-z-D-glucopyranosyl diacylglycerols. The major fatty acids (>10%) are summed feature 7 (one or more of C<sub>18:1</sub>ω7c, C<sub>18:1</sub>ω9t and C<sub>18:1</sub>ω12t) and C<sub>16:0</sub>. The minor fatty acids are C<sub>19:0</sub> cyclo ω8c, summed feature 4 (one or more of iso-C<sub>15:0</sub>:2-OH, C<sub>16:1</sub>ω7c and C<sub>16:1</sub>ω7t), C<sub>15:0</sub>, C<sub>12:0</sub>, 3-OH, C<sub>17:1</sub>ω8c, C<sub>14:0</sub>, 11-methyl C<sub>18:1</sub>ω9t and C<sub>17:0</sub>. The G+C content of the type strain is 66.3 mol%.

The type strain is LMG 2337<sup>T</sup> (=CCUG 1797<sup>T</sup> =ATCC 14736<sup>T</sup>), isolated from blood of a patient with endocarditis.

**Acknowledgements**

We thank Marc Vancanneyt for his contribution to the cellular fatty acid analyses. We are indebted to Dagmar Wenderoth and Peter Wolff for their excellent technical assistance. This work was supported by grants of the European Union within the T-project ‘High Resolution Automated Microbial Identification and Application to Biotechnologically Relevant Ecosystems’ and the German Federal Ministry for Science, Education and Research (projects no. 031943SC and 01KI 07 96).

**References**


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